REMARKS

Reconsideration of the present application in view of the above amendments and following remarks is respectfully requested. Claims 1, 2, 4, 12, 13, 15-18, 20, and 29-53 were pending. (Please note that claim 19 was previously canceled without prejudice in response to Restriction Requirement filed on February 9, 2001 (Paper 10); however, the instant Office Action shows claim 19 as still pending; Applicants confirm that claim 19 is to be canceled without prejudice). As set forth above, Applicants hereby cancel claims 30, 33, 34, 38, 39, 43, and 46 without prejudice to the filing of any divisional, continuation, or, continuation-in-part application. Applicants hereby submit new claims 54-64. Support for new claims may be found in the specification, in part, at page 27, lines 16-18, at page and Figure 6 (*see, e.g.*, claim 54); Example 2 (*see, e.g.*, claim 55); at page 26, lines 10-22 and Figure 6 (*see, e.g.*, claims 56 and 57); at page 11, lines 14-17 and Example 10 (*see, e.g.*, claims 61 and 62); and at page 22, line 17 through page 25, line 17 (*see, e.g.*, claims 58-64). Claims 1, 20, 29, 40-42, and 50 have been hereby amended to more clearly define the subject matter encompassed by Applicants' invention, and claims 32, 35, 44, 47, and 51 have been amended for mere editorial purposes to correct claim dependencies and/or to add sequence identifiers. Support for amended claims may be found in the subject application as originally filed, in part, in Table 2, at page 28-29; in Example 7 at page 47; in Example 9 at page 52; and in Figure 7 (*see, e.g.*, claims 29, 41, and 42); and at page 22, lines 1-8; and at page 27, lines 8-10 (*see, e.g.*, claim 46). No new matter has been added. Therefore, claims 1, 2, 4, 12, 13, 15-18, 20, 29, 31, 32, 35-37, 40-42, 44, 45, and 47-64 are currently pending.

Applicants note that reference AD (U.S. Patent No. 6,242,219, Better) of the Second Supplemental Information Disclosure Statement filed on November 27, 2001 was inadvertently not initialed as considered by the Examiner. Applicants respectfully request that this reference be considered. Applicants enclose a copy of reference AD for the Examiner's convenience.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The first page of the attached pages is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

DRAWINGS

In the Office Action dated March 8, 2002, the drawings, as filed with the subject application on November 19, 1999, are objected to for the reasons cited in the Notice of Draftperson's Patent Drawing Review (PTO Form 948). Applicants respectfully submit that formal drawings are being provided herewith to address all alleged deficiencies with the originally filed drawings. Accordingly, Applicants respectfully submit that this ground of objection has been obviated and request that it be withdrawn.

DECLARATION UNDER 37 C.F.R. §1.131

In the Office Action, it is alleged that the Exhibit provided with the Declaration of Burian and Bartfeld filed under 37 C.F.R. §1.131 (filed December 17, 2001) fails to provide evidence that the disclosed nucleic acid constructs include sequence encoding an anionic spacer. Therefore, it is alleged that the Exhibit fails to prove that anionic spacers as claimed in the instant invention were contemplated prior to 1998.

Applicants respectfully traverse this finding and submit that the Exhibit filed December 17, 2001 with the Rule 131 Declaration clearly teaches that "anionic spacers" were used in the disclosed nucleic acid constructs to express multiple copies of cationic peptides. Applicants note that on page 1 of the Exhibit, it is stated that the spacer used "is a natural sequence from Apis mellifera (honeybee); it occurs between identical copies of genes producing apidaecins." As is known in the art and as described in the specification, apidaecin is an example of a cationic peptide (*see, e.g.*, specification at page 14, lines 11-14), and the naturally occurring "spacers" found between apidaecins are anionic (*see, e.g.*, specification at page 20, line 30 through page 21, line 4 and reference cited therein, Casteels-Josson *et al.*, *EMBO J. 12*:1569, 1993, abstract enclosed herewith). Therefore, a person having ordinary skill in the art would appreciate that Applicants explicitly disclose "anionic spacers" and, in fact, actually used sequences that encode "anionic spacers" in nucleic acid expression constructs according to the instant invention.

Accordingly, Applicants respectfully request that the Declaration of Burian and Bartfeld and Exhibit attached thereto, filed under 37 C.F.R. §1.131 on December 17, 2001, be reconsidered. Furthermore, in view of the reconsidered Declaration and Exhibit, Applicants

respectfully submit that Zhang *et al. (Biochem. Biophys Res. Comm. 247*:674-680, 1998) is not properly prior art to the instant claimed invention.

REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH

(1) In the Office Action, claims 29, 33, 37, 38 and 40 were rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. In particular, it is alleged that the claimed expression cassettes are infinitely broad with regard to the number and position of the cassette components. In addition, it is asserted that the teachings of Zhang *et al. (Biochem. Biophys Res. Comm. 247*:674-680, 1998) show that the sequence and position of each component is crucial for successful expression of cationic peptides. Hence, it is alleged that the instant specification only provides guidance for multi-domain fusion proteins wherein the cationic peptide is an indolicidin sequence, the carrier protein is cellulose binding domain, the anionic spacer is SEQ ID NO:27, and only when these components are in a particular order. Therefore, it is asserted that in light of the prior art and guidance in the instant specification, a person having ordinary skill in the art would be required to experiment unduly to successfully make and use the full scope of the claimed invention.

Applicants respectfully traverse this ground of rejection and submit that the disclosure of the instant specification is commensurate with the scope of claims and that no undue experimentation is required to practice the invention. As an initial matter, Applicants do not understand how a specific fusion protein structure and a specific number of components, as recited in original claim 29, could "indicate that the number and positions of any of these components is almost unlimited" for the claimed fusion expression constructs (*see* Office Action, paragraph 15, last line of page 6 through line 5 of page 7). Nonetheless, merely to expedite prosecution of the subject application, Applicants have amended claim 29 to more clearly define the claimed invention. In particular, the instant claimed invention is directed, in pertinent part, to a multi-domain fusion protein expression cassette, comprising a promoter operably linked to a nucleic acid molecule that encodes a fusion protein, wherein the encoded fusion protein comprises a structure of [(cleavage site)-(indolicidin analog)-(cleavage site)-(anionic spacer peptide)]$_n$ with n being an integer having a value between 1 and 40 and wherein at least one indolicidin analog has antimicrobial activity. As taught by the instant specification, a fusion

protein having an equal number (n) of indolicidin analogs and anionic spacer peptides in alternating positions is expressed very well, with or without a carrier amino acid sequence (*see, e.g.*, specification, Table 2 at page 29). Concededly, the presence of a carrier amino acid sequence is optional for expression and, more importantly, the sequence of the carrier was irrelevant to the claimed expression constructs as a cellulose binding domain of 180 amino acids (*see, e.g.*, specification, Example 1; *see also* Exhibit attached to Declaration filed under 37 C.F.R. §1.131 on December 17, 2001, at page 4 of 23) and of 96 amino acids (*see, e.g.*, specification, Table 2 at page 29) were both made and used to successfully express multi-domain fusion proteins comprising indolicidin analogs according to the instant invention. Furthermore, Applicants submit that the instant specification provides ample guidance as to the size, structure, and type of carrier amino acid sequence to use in the instant invention (*see, e.g.*, specification, at page 21, lines 15-28). Thus, in contrast to the teachings of Zhang *et al.*, whether a carrier amino acid sequence is present and the exact structure of such a carrier is irrelevant to the claimed multi-domain fusion protein expression cassettes.

In addition, the specification teaches that a fusion protein having n indolicidin analogs and n-2 anionic spacers with a structure of [(cleavage site)-(indolicidin analog)-(cleavage site)-(anionic spacer peptide)]$_n$-(cleavage site)-(indolicidin analog)-(cleavage site)-(indolicidin analog) is expressed very well according to the instant invention (*see, e.g.*, specification, Table 2 at page 29; and new claim 54). Similarly, the specification teaches the construction of a fusion protein having n cationic peptides (*i.e.*, MBI 26, which is a cecropin-mellitin hybrid peptide) and n-1 anionic spacers (*see, e.g.*, specification, Example 8, Part B at page 51, lines 10-11), which Applicants found to be expressed. In this regard, Applicants have made of record evidence showing that a fusion protein expression construct made with a structure of n indolicidin analogs and n-1 anionic spacers (*i.e.*, a structure of (carrier amino acid)-(cleavage site)-(indolicidin analog)-(cleavage site)-(anionic spacer peptide)-(cleavage site)-(indolicidin analog)) and used according to the instant invention is also expressed well (*see, e.g.*, Exhibit attached to the Declaration filed under 37 C.F.R. §1.131 on December 17, 2001, Figure at the bottom of page 1 of 23, and pages 9-11 of 23; and new claim 54). Moreover, Applicants respectfully submit that a variety of different indolicidin analogs and different anionic peptide spacers have been used to construct multi-domain fusion protein expression cassettes according to the instant invention,

which were successfully used to express the various fusion proteins (a Declaration under Rule 132 in support of such results will be forthcoming). Again, in contrast to Zhang *et al.*, the sequence and exact position of each component is not crucial for successful expression of fusion proteins according to the instant invention. Hence, the instant specification provides a person having ordinary skill in the art ample guidance to make and use the instant invention commensurate with the scope of the claims and without undue experimentation.

Accordingly, Applicants respectfully submit that the claims satisfy the requirements of 35 U.S.C. §112, first paragraph and, therefore, request that this rejection be withdrawn.

(2) In the Office Action, claims 30-32, 34-36, 39 and 41-53 were rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. In particular, it is alleged that these dependent claims only add limitations directed to location, to identity or structure of individual components, and to the number of cationic peptides present in the fusion protein. Therefore, for the reasons stated above, it is asserted that in light of the prior art and guidance in the instant specification, a person having ordinary skill in the art would be required to experiment unduly to successfully make and use the full scope of the claimed invention.

Applicants respectfully traverse this ground of rejection and submit that the disclosure of the instant specification is commensurate with the scope of claims and that no undue experimentation is required to practice the invention. As an initial matter, Applicants respectfully submit that this rejection with regard to claims 30, 34, 39, 43, and 46 is rendered moot because these claims have been hereby canceled without prejudice. With regard to the remaining claims, Applicants submit that within the scope of the instant invention (*see, e.g.*, claim 29), any carrier may be used, a variety of anionic spacers may be used, and a variety of indolicidin analogs may be used, as set forth in Part (1) above. Therefore, Applicants respectfully submit that because the full scope of independent claim 29 is enabled by the instant specification, then it follows that each dependent claim is also necessarily enabled.

Accordingly, Applicants respectfully submit that the claims satisfy the requirements of 35 U.S.C. §112, first paragraph and, therefore, request that this rejection be withdrawn.

REJECTIONS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

(1) In the Office Action, claims 29-53 were rejected under 35 U.S.C. §112, second paragraph as indefinite. In particular, it is alleged that claim 29 is unclear and confusing with regard to the position of the cleavage site. In addition, it is alleged that claim 33 fails to further limit claim 29. Claim 34 is allegedly confusing with regard to the location of "the carrier." Claim 44 is allegedly indefinite for lacking a sequence identifier. Claim 46 is allegedly unclear with regard to the phrase "the cleavage site is with the...".

Applicants respectfully traverse these grounds of rejection. As an initial matter, Applicants respectfully submit that the rejections of claims 33, 34, and 46 are rendered moot because these claims have been, merely to expedite prosecution and not due to any acquiescence regarding these rejections, hereby canceled without prejudice. With regard to claim 44, Applicants thank the Examiner for identifying the inadvertent oversight of not reciting the sequence identifiers for the recited cationic peptides, and submit that this rejection is now rendered moot as well because claim 44 has been amended to include the respective sequence identifiers. Finally with regard to original claim 29, Applicants submit that the position of the cleavage site is clear in view of the recited fusion protein structure. However, merely to expedite prosecution of the subject application, Applicants have amended claim 29 to incorporate the original description of the cleavage sites directly into the recited structure. Therefore, Applicants respectfully submit that the metes and bounds of claim 29, as amended, is sufficiently clear for a person having ordinary skill in the art.

Accordingly, Applicants respectfully submit that the invention as presently claimed satisfies the requirements of 35 U.S.C. §112, second paragraph and, therefore, request that these rejections be withdrawn.

REJECTION UNDER U.S.C. §102(e)

In the Office Action, claims 1, 2, 4, 16, 17, 18, and 20 were rejected under 35 U.S.C. §102(e) as anticipated by U.S. Patent No. 5,851,802 (Better). In particular, it is asserted that Better provides a fusion protein expression cassette comprising a nucleic acid molecule that encodes a polypeptide having the structure (cationic peptide)-[(cleavage site)-

(cationic peptide)]$_n$ wherein n has a value up to 4, as claimed. The cationic peptide disclosed by Better is the antimicrobial peptide BPI.

Applicants respectfully traverse this ground of rejection and submit that Better fails to meet every limitation of the instant claim and, therefore, fails to anticipate the claimed invention. As described in the specification and recited in the amended claims, the instant invention is directed, in pertinent part for this rejection, to a fusion protein expression cassette, comprising a promoter operably linked to a nucleic acid molecule that encodes an indolicidin analog fusion protein, wherein the encoded fusion protein comprises a structure of (indolicidin analog)-[(cleavage site)-(indolicidin analog)]$_n$ with *n* being an integer having a value between one and three and wherein the indolicidin analogs have antimicrobial activity. Better merely describes nucleic acid expression constructs for BPI peptides only and is silent with regard to any other cationic peptide. Thus, Better fails to teach or suggest a fusion protein construct including an indolicidin analog according to the instant invention.

Accordingly, Applicants respectfully request that this rejection under 35 U.S.C. §102(e) be withdrawn because the instant claims are patentably distinct over Better.

REJECTION UNDER U.S.C. §103(a)

In the Office Action, claim 15 was rejected under 35 U.S.C. §103(a) as being unpatentable over Better (U.S. Patent 5,851,802) in view of Zhang *et al. (Biochem. Biophys Res. Comm. 247*:674-680, 1998). In particular, it is asserted that it would have been obvious for a person having ordinary skill in the art to modify the expression vectors of Better to include the T7 promoter of Zhang *et al.*

Applicants respectfully traverse this ground of rejection and submit that Better and Zhang *et al.*, taken alone or in combination, fail to teach or suggest the claimed invention. As noted above, Applicants have requested that the Declaration and Exhibit filed under 37 C.F.R. §1.131 on December 17, 2001 be reconsidered in view of the explicit disclosure of fusion protein expression constructs having anionic spacers. Therefore, as previously made of record, Applicants have reviewed laboratory records and readily conclude that compositions of matter and methods as claimed in the subject application were conceived prior to 1998 (*i.e.*, prior

to the publication of Zhang *et al.*). Consequently, Applicants respectfully submit that Zhang *et al.* has been removed as citable prior art and that this rejection has been rendered moot.

Accordingly, in view of the pending claims and the foregoing remarks, Applicants respectfully submit that this rejection under 35 U.S.C. §103(a) have been overcome and request that this rejection be withdrawn.

All of the claims pending in the application are now clearly allowable. Favorable consideration and a Notice of Allowance are earnestly solicited. The Examiner is urged to contact the undersigned attorney if there are any questions prior to allowance of this matter.

Respectfully submitted,

Seed Intellectual Property Law Group PLLC

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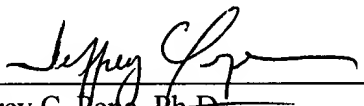
PATENT TRADEMARK OFFICE



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Enclosures:

16 sheets of Formal Drawings (Figs. 1A – 11)
Reference AD

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Claims 30, 33, 34, 38, 39, 43, and 46 have been canceled without prejudice.

All pending claims 1, 2, 4, 12, 13, 15-18, 20, 29, 31, 32, 35-37, 40-42, 44, 45, and 47-64 are provided for the Examiner's convenience. Claims 1, 20, 29, 32, 35, 40-42, 44, 47, 50, and 51 have been amended, and claims 54-64 have been added, as follows:

1. (Twice Amended) A ~~multi-domain~~ fusion protein expression cassette, comprising a promoter operably linked to a nucleic acid molecule ~~which is expressed as an insoluble protein, wherein said nucleic acid molecule encodes a polypeptide comprising the structure (cationic peptide)-[(cleavage site)-(cationic peptide)]$_n$, wherein n is an integer having a value between one and four and at least one cationic peptide has antimicrobial activity~~ that encodes an indolicidin analog fusion protein, wherein the encoded fusion protein comprises a structure of (indolicidin analog)-[(cleavage site)-(indolicidin analog)]$_n$ with n being an integer having a value between one and three and wherein the indolicidin analogs have antimicrobial activity.

2. The expression cassette according to claim 1 wherein said nucleic acid molecule also encodes a carrier protein.

4. The expression cassette according to any one of claims 1 or 2 wherein said cleavage site can be cleaved by low pH or by a reagent selected from the group consisting of cyanogen bromide, N-chlorosuccinimide, 2-(2-nitrophenylsulphenyl)-3-methyl-3'-bromoindolenine, hydroxylamine, *o*-iodosobenzoic acid, Factor Xa, Factor XIIa, thrombin, enterokinase, collagenase, *Staphylococcus aureus* V8 protease, endoproteinase Arg-C, endoproteinase Glu-C, endoproteinase Lys-C, and trypsin.

12. The expression cassette according to claim 2 wherein said carrier protein is less than 100 amino acid residues in length.

13. The expression cassette according to claim 2 wherein said carrier protein is a truncated cellulose binding domain of less than 100 amino acids.

15. The expression cassette according to any one of claims 1 or 2 wherein said promoter is selected from the group consisting of *lacP* promoter, *tacP* promoter, *trcP* promoter, *srpP* promoter, SP6 promoter, T7 promoter, *araP* promoter, *trpP* promoter, and λ promoter.

16. A recombinant host cell comprising the expression cassette according to any one of claims 1, 2, 12, or 13.

17. The recombinant host cell of claim 16 wherein said host cell is a yeast, fungi, bacterial or plant cell.

18. The recombinant host cell of claim 17 wherein said bacterial host cell is *Escherichia coli*.

20. (Twice Amended) A method of producing ~~fusion proteins that contain a cationic peptide~~ a fusion protein that contains at least one indolicidin analog, comprising culturing the recombinant host cell of claim 16 under conditions and for a time sufficient to produce said fusion protein.

29. (Amended) A multi-domain fusion protein expression cassette, comprising a promoter operably linked to a nucleic acid molecule ~~that is expressed as an insoluble protein, wherein the nucleic acid molecule encodes a fusion protein comprising (a) a carrier amino acid sequence, (b) an anionic spacer peptide, (c) at least two cationic peptides wherein at least one cationic peptide has antimicrobial activity, and (d) at least two cleavage sites wherein at least one cleavage site is between the cationic peptide and the carrier and at least one~~

~~cleavage site is between the cationic peptide and the spacer, wherein the encoded fusion protein comprises the structure (carrier amino acid sequence) [(cationic peptide)-(anionic spacer peptide)]$_n$-(cationic peptide) with n being an integer having a value between 1 and 100~~ that encodes a fusion protein, wherein the encoded fusion protein comprises a structure of [(cleavage site)-(indolicidin analog)-(cleavage site)-(anionic spacer peptide)]$_n$ with n being an integer having a value between 1 and 40 and wherein the indolicidin analogs have antimicrobial activity.

31. The expression cassette according to claim 29 wherein the promoter is selected from the group consisting of *lacP* promoter, *tacP* promoter, *trcP* promoter, *srpP* promoter, SP6 promoter, T7 promoter, *araP* promoter, *trpP* promoter, and λ promoter.

32. (Amended) The expression cassette according to ~~claim 29~~ claim 55 wherein the carrier is selected from cellulose binding domain, glutathione-S-transferase, outer membrane protein F, β-galactosidase, protein A, or IgG-binding domain.

35. (Amended) The expression cassette according to ~~claim 29~~ claim 55 wherein the carrier is less than 100 amino acid residues in length.

36. The expression cassette according to claim 35 wherein the carrier is a truncated cellulose binding domain.

37. The expression cassette according to claim 29 wherein the anionic spacer has no cysteine residue.

40. (Amended) The expression cassette according to claim 29 wherein the cumulative charge of the anionic spacer peptide reduces the cumulative charge of the ~~cationic peptide~~ indolicidin analog.

41. (Amended) The expression cassette according to claim 29 wherein the fusion protein comprises from ~~2 to 40 cationic peptides~~ about 5 to about 30 indolicidin analogs.

42. (Amended) The expression cassette according to claim 29 wherein the fusion protein comprises from ~~2 to 20 cationic peptides~~ about 10 to about 20 indolicidin analogs.

44. (Amended) The expression cassette according to ~~claim 43~~ any one of claims 1 or 29 wherein the ~~indolicidin or analog thereof is an~~ indolicidin analog ~~of~~ has up to 35 amino acids ~~that comprises~~ comprising the sequence of ~~I L K K W P W W P W R R K or I L R W P W W P W R R K~~SEQ ID NO:35 or SEQ ID NO:36.

45. The expression cassette according to claim 29 wherein the cleavage site can be cleaved by low pH or by a reagent selected from cyanogen bromide, N-chlorosuccinimide, 2-(2-nitrophenylsulphenyl)-3-methyl-3'-bromoindolenine, hydroxylamine, *o*-iodosobenzoic acid, Factor Xa, Factor XIIa, thrombin, enterokinase, collagenase, *Staphylococcus aureus* V8 protease, endoproteinase Glu-C, endoproteinase Arg-C, endoproteinase Lys-C, chymotrypsin, trypsin, or a combination thereof.

47. (Amended) A recombinant host cell comprising the expression cassette according to any one of ~~claims 29-46~~ claims 29, 37, 41, or 42.

48. The recombinant host cell of claim 47 wherein the host cell is a yeast, a fungus, a bacteria or a plant cell.

49. The recombinant host cell of claim 48 wherein the bacteria is *Escherichia coli*.

50. (Amended) A method of producing ~~fusion proteins that contain a cationic peptide~~ a fusion protein that contains at least one indolicidin analog, comprising culturing the recombinant host cell of claim 47 under conditions and for a time sufficient to produce the fusion protein.

51. (Amended) The expression cassette according to any one of claims 1, 2, 29, or ~~30~~ 54 wherein the expression cassette is contained in an expression vector.

52. The recombinant host cell of claim 16 wherein the expression cassette is contained in an expression vector.

53. The recombinant host cell of claim 47 wherein the expression cassette is contained in an expression vector.

54. (New) The expression cassette according to claim 29 further consisting of one additional indolicidin analog or two additional indolicidin analogs, wherein the additional analog or analogs are at the carboxy-terminus of the encoded fusion protein.

55. (New) The expression cassette according to any one of claims 29 or 54 further comprising a carrier amino acid sequence wherein the carrier amino acid sequence is at the amino-terminus of the encoded fusion protein.

56. (New) The expression cassette according to any one of claims 1, 29, or 54 wherein the indolicidin analog is SEQ ID NO:36.

57. (New) The expression cassette according to claim 55 wherein the indolicidin analog is SEQ ID NO:36.

58. (New) The recombinant host cell according to claim 53 wherein the encoded indolicidin analog fusion protein is expressed as an insoluble protein.

59. (New) A recombinant host cell comprising the expression cassette according to claim 57 wherein the expression cassette is contained in an expression vector.

60. (New) A recombinant host cell comprising the expression cassette according to claim 58 wherein the expression cassette is contained in an expression vector.

61. (New) The recombinant host cell according to claim 59 wherein the encoded indolicidin analog fusion protein is expressed as an insoluble protein.

62. (New) The recombinant host cell according to claim 60 wherein the encoded indolicidin analog fusion protein is expressed as an insoluble protein.

63. (New) A method of producing a fusion protein that contains at least one indolicidin analog, comprising culturing a recombinant host cell according to claim 59 under conditions and for a time sufficient to produce said fusion protein.

64. (New) A method of producing a fusion protein that contains at least one indolicidin analog, comprising culturing a recombinant host cell according to claim 60 under conditions and for a time sufficient to produce said fusion protein.